

Amendments to the Specification

Please replace the pending paragraph beginning on page 51, line 29 with the following paragraph:

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In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (*e.g.*, IL-2, IL-12, GM-CSF), cytokine-inducing molecules (*e.g.*, LeIF), costimulatory molecules, or for HTL responses, pan-DR binding proteins (*e.g.*, PADRE[®] epitopes, Epimmune, San Diego, CA). Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving HTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (*e.g.*, TGF- β) may be beneficial in certain diseases.

Please replace the pending paragraph beginning on page 54, line 26 with the following paragraph:

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Alternatively, it is possible to prepare synthetic peptides capable of stimulating T helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in

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nature (*see, e.g.*, PCT publication WO 95/07707). These synthetic compounds called Pan-DR-binding epitopes (*e.g.*, PADRE® epitopes, Epimmune, Inc., San Diego, CA) are designed to most preferably bind most HLA-DR (human HLA class II) molecules. For instance, a pan-DR-binding epitope peptide having the formula: aKXVAAWTLKAAa (SEQ ID NO:6698), where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and "a" is either D-alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals, regardless of their HLA type. An alternative of a pan-DR binding epitope comprises all "L" natural amino acids and can be provided in the form of nucleic acids that encode the epitope.

Please replace the pending paragraph beginning on page 82, line 22 with the following paragraph:

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Assay for cytotoxic activity: Target cells (1.0 to 1.5×10^6) are incubated at 37°C in the presence of $200 \mu\text{l}$ of ^{51}Cr . After 60 minutes, cells are washed three times and resuspended in medium. Peptide is added where required at a concentration of $1 \mu\text{g/ml}$. For the assay, 10^4 ^{51}Cr -labeled target cells are added to different concentrations of effector cells (final volume of $200 \mu\text{l}$) in U-bottom 96-well plates. After a 6 hour incubation period at 37°C , a 0.1 ml aliquot of supernatant is removed from each well and radioactivity is determined in a Micromedic Micromedic® automatic gamma counter. The percent specific lysis is determined by the formula: percent specific release = $100 \times (\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})$. To facilitate comparison between separate CTL assays run under the same conditions, % ^{51}Cr release data is expressed as lytic units/ 10^6 cells. One lytic unit is arbitrarily

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defined as the number of effector cells required to achieve 30% lysis of 10,000 target cells in a 6 hour ⁵¹Cr release assay. To obtain specific lytic units/10⁶, the lytic units/10⁶ obtained in the absence of peptide is subtracted from the lytic units/10⁶ obtained in the presence of peptide. For example, if 30% ⁵¹Cr release is obtained at the effector (E): target (T) ratio of 50:1 (i.e., 5x10⁵ effector cells for 10,000 targets) in the absence of peptide and 5:1 (i.e., 5x10⁴ effector cells for 10,000 targets) in the presence of peptide, the specific lytic units would be: [(1/50,000)-(1/500,000)] x10⁶ = 18 LU.

Please replace the pending paragraph beginning on page 85, line 14 with the following paragraph:

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Overlapping oligonucleotides that can, for example, average about 70 nucleotides in length with 15 nucleotide overlaps, are synthesized and HPLC-purified. The oligonucleotides encode the selected peptide epitopes as well as appropriate linker nucleotides, Kozak sequence, and signal sequence. The final multiepitope minigene is assembled by extending the overlapping oligonucleotides in three sets of reactions using PCR. A Perkin/Elmer Perkin/Elmer® 9600 PCR machine is used and a total of 30 cycles are performed using the following conditions: 95°C for 15 sec, annealing temperature (5° below the lowest calculated T_m of each primer pair) for 30 sec, and 72°C for 1 min.

Please replace the pending paragraph beginning on page 85, line 22 with the following paragraph:

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For example, a minigene can be prepared as follows. For a first PCR reaction, 5 µg of each of two oligonucleotides are annealed and extended: In an example using eight oligonucleotides, *i.e.*, four pairs of primers, oligonucleotides 1+2, 3+4, 5+6, and 7+8 are combined in 100 µl reactions containing *Pfu* polymerase buffer (1x= 10 mM KCL, 10 mM (NH₄)₂SO₄, 20 mM Tris-chloride, pH 8.75, 2 mM MgSO₄, 0.1% Triton X-100, 100 µg/ml BSA), 0.25 mM each dNTP, and 2.5 U of *Pfu* polymerase. The full-length dimer products are gel-purified, and two reactions containing the product of 1+2 and 3+4, and the product of 5+6 and 7+8 are mixed, annealed, and extended for 10 cycles. Half of the two reactions are then mixed, and 5 cycles of annealing and extension carried out before flanking primers are added to amplify the full length product. The full-length product is gel-purified and cloned into Invitrogen® plasmid pCR-blunt (~~Invitrogen~~) and individual clones are screened by sequencing.

Please replace the pending paragraph beginning on page 91, line 25 with the following paragraph:

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PBMC from vaccinated individuals are separated on ~~Ficoll-Histopaque~~ Ficoll-Histopaque® density gradients (Sigma Chemical Co., St. Louis, MO), washed three times in HBSS (GIBCO Laboratories), resuspended in RPMI-1640 (GIBCO Laboratories) supplemented with L-glutamine (2mM), penicillin (50U/ml), streptomycin (50 µg/ml), and Hepes (10mM) containing 10% heat-inactivated human AB serum (complete RPMI) and plated using microculture formats. A synthetic peptide comprising an epitope of the invention is added at 10 µg/ml to each well and HBV core 128-140 epitope is added at 1 µg/ml to each well as a source of T cell help during the first week of stimulation.

Please replace the pending page 108 with the attached replacement page 108.

Please cancel the existing Sequence Listing for the above-identified application, replace it with the substitute Sequence Listing appended hereto, and insert the same at the end of the application.